TUBERCULOSIS-RELATED RESEARCH

at the

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

March 1998

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Keppel Street
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TUBERCULOSIS-RELATED RESEARCH PROJECTS 1998

To obtain further copies of this document and further information on our Tuberculosis-related research please contact:

Sarah Lewin
Clinical Research

Tel: 0171 927 2194
Fax: 0171 637 4314
E-mail: S.lewin@lshtm.ac.uk

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Abbreviations for the London School's Academic Departments

Epidemiology & Population Health	**(EPH)**
Infectious and Tropical Diseases	**(ITD)**
Public Health & Policy	**(PHP)**

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LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A randomized placebo-controlled intervention study to evaluate the safety and effectiveness of trimethoprim-sulfamethoxazole in reducing mortality in HIV-infected tuberculosis patients in Abidjan, Côte d'Ivoire

LSHTM STAFF: Alison Grant, Kevin de Cock (ITD)

COLLABORATORS: Project RETRO-CI, Abidjan, Cote D'Ivoire

FUNDING: Centers for Disease Control and Prevention, Atlanta, USA; Roche African Research Foundation; Rockefeller Foundation

SUMMARY:

LSHTM is involved in the part of this study which aims to determine the causes of morbidity and mortality among HIV-infected patients with tuberculosis.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Tuberculosis and HIV studies in Nairobi, Kenya - I

LSHTM STAFF: Mark Hawken, John Porter (ITD)

COLLABORATORS: KEMRI;
University of Nairobi;
Wellcome Trust Research Laboratories, Kenya

FUNDING: Rockefeller Foundation

SUMMARY:

This study was performed to assess the presentation, HIV seroprevalence and outcome of patients admitted to the acute medical service at Kenyatta National Hospital, the main government referral hospital in Nairobi. Running in parallel with this was a post-mortem study to describe the range of pathology in HIV-infected patients that die in hospital. 1780 new admissions were studied over an 11 month period. 1540 (86.5%) underwent HIV testing, of whom 42.5% were HIV-1 positive.

Seventy HIV-1 positive autopsies were performed. Provisional results show tuberculosis and bacterial pneumonia cause 75% of all HIV-1 positive deaths. The tuberculosis was disseminated in 93% of cases. The median CD4 count on admission was $20x10^6/l$ in those HIV-1 positive patients who died in the ward.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Tuberculosis and HIV studies in Nairobi - II

LSHTM STAFF: Mark Hawken, John Porter (ITD)

COLLABORATORS: KEMRI;
University of Nairobi;
Wellcome Trust Research Laboratories, Kenya

FUNDING: DFID

SUMMARY:

This study began in October 1997 to determine the true ætiology of "smear-negative presumed tuberculosis" and so establish the proportion of this disease entity that is not tuberculosis (but some other HIV-related pulmonary disease). This will provide the necessary information to develop a more rational, cost-effective diagnostic work-up in countries with a high prevalence of HIV and tuberculosis.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Drug resistance survey and transmission patterns of tuberculosis in refugee and non-refugee populations in Kenya: Analysis by molecular methods

LSHTM STAFF: Willie Abela Githui (ITD)
Mark Hawken, John Porter, Peter Godfrey-Faussett (ITD)

COLLABORATORS: E Juma KEMRI; D Kibuga, Kenya National Leprosy and Tuberculosis programme; B Swai, United Nations High Commission for Refugees; F Drobniewski, Mycobacterium Reference Laboratory, Dulwich

FUNDING: PhD Project

SUMMARY

Sputum microscopy, culture, strain identification and drug susceptibility assays were carried out according to standard techniques. Strains identified as *M. tuberculosis* and with susceptibility results were analysed using IS6110 based Restriction Fragment Length Polymorphism (RFLP) technique. Results indicated high rates of resistance in the refugee camps compared to those at the Manyatta treatment center. Multidrug resistant tuberculosis (MDR-TB) was also predominant in the camps. IS6110 based RFLP analysis was inconclusive in over 50% of isolates analysed. A second marker is necessary to further characterise these isolates.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Epidemiology of tuberculosis in northern Malawi

LSHTM STAFF: Paul Fine, Judith Glynn David Warndorff, Hazel Dockrell, Philip Broadbent , Keith Branson, Jane Bruce, Lyn Bliss, Gillian Black (ITD)

COLLABORATORS: Bagrey Ngwira (Karonga Prevention Study) Francis Drobniewski (PHLS, Dulwich), Sebastian Lucas (UMDS) Adrian Hill (Oxford) Jenefer Blackwell (Cambridge) and others

FUNDING: Wellcome Trust Programme Grant. LEPRA

SUMMARY:

Tuberculosis surveillance has been carried out actively and passively in Karonga district in northern Malawi since 1985. Two total population surveys carried out in the 1980s provide detailed background data on the population (currently estimated at 180,000 people) including demographic characteristics, household location, family relationships, and various socio-economic indicators. Tuberculin skin tests were carried out on almost 100,000 individuals and more than 40,000 blood specimens were collected and stored. All isolates of *M. tuberculosis* have been screened for drug resistance and those collected since 1991 have been fingerprinted (IS6110). Since 1988 all cases have been HIV tested, along with age-sex-area matched controls. A vaccine trial was initiated in 1986 to evaluate the efficacy of repeat BCG and of BCG and killed *M. leprae* in protecting against tuberculosis (and leprosy). A first report has been published but follow-up continues (supported by LEPRA).

Current work on the collected data includes analyses of skin test results, studies of outcome of tuberculosis, analysis of the case-control study of relationships with HIV, analyses of risk factors for tuberculosis, analysis of atypical mycobacteria, analysis of RFLP results.

In 1996 we were awarded a Wellcome Trust Programme grant for new research projects in this area. The new areas covered include:

(i) Genetic risk factors for mycobacteria, making use of our database to identify related individuals and using new case-control studies

(ii) Immunological correlates of protective immunity to mycobacterial diseases using skin tests and cytokine responses to a range of mycobacterial antigens

(iii) Patterns of transmission of tuberculosis using RFLP and the epidemiological relationships recorded in our database and determined from questionnaires to new patients.

(iv) Risk factors for tuberculosis, and relationships with HIV, based on continuing case-controls studies and a retrospective cohort study of HIV.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A randomised control trial of single-dose of *M vaccae* in the immunotherapy of (HIV positive) pulmonary tuberculosis

LSHTM STAFF: Paul Fine, David Warndorff, Judith Glynn, Lyn Bliss, Mia Crampin (ITD)

COLLABORATORS: Dr Bagrey Ngwira, Karonga Prevention Study; Dr Andrew Nunn and Dr Janet Darbyshire, MRC HIV Clinical Trials Unit; Dr Ali Zumla, UCLMS

FUNDING: DFID

SUMMARY:

This is a collaborative trial carried out in Karonga District, Northern Malawi and at the University Teaching Hospital in Lusaka, Zambia.

250 culture positive tuberculosis cases will be recruited in Karonga District and randomised to receive a single injection of either killed *M vaccae* or placebo one week after starting standard chemotherapy. They will be kept in hospital until smear negative, and followed up monthly for two years. Major outcome variables include mortality, rate of conversion to sputum negativity, and relapse.

LSHTM TUBERCULOSIS – RELATED RESEARCH

TITLE: Operational research on tuberculosis in Malawi

LSHTM STAFF: Judith Glynn (ITD)

COLLABORATORS: Tony Harries (Malawi)

FUNDING: DFID, WHO

SUMMARY:

Tony Harries is funded by the DFID to carry out operational research on TB in Malawi including evaluation of treatment regimens, diagnostic procedures, decentralisation of services, integration of TB, HIV and STD control, and TB in prisons. The School will provide epidemiological guidance and help with analyses. So far this has focused on smear negative pulmonary tuberculosis is HIV positive patients.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Ecology and implications of environmental mycobacteria in Karonga District, northern Malawi

LSHTM STAFF: Ben Chilima (PhD student), Paul Fine (ITD)

COLLABORATORS: Dr Penny Hirsch, Rothamsted ARC; Dr F Drobniewski, Dulwich PHLS

FUNDING: EC

SUMMARY:

BCG has been found to provide no detectable protection against tuberculosis in northern Malawi. One explanation for this observation is that extensive exposure to environmental mycobacteria in that environment has provided as much protection as can be provided by BCG. This project seeks to identify and characterise the environmental mycobacterial flora in Karonga District, and to relate this to skin test sensitivity patterns and isolates obtained in sputum samples. The work includes development of new DNA probe methods for identifying mycobacteria in soil samples.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Studies of mycobacterial infections in South African goldminers

LSHTM STAFF: Dr Kevin De Cock, Dr Liz Corbett (ITD)

COLLABORATORS:

FUNDING: The Wellcome Trust

SUMMARY:

This study aims to investigate the effects of HIV infection on mycobacterial disease in South African gold miners, a population with high pre-HIV prevalence rates of tuberculosis, disease due to mycobacteria other than TB (MOTT) and occupational lung disease.

The magnitude of HIV infection as a risk factor for TB and MOTT disease will be investigated retrospectively using a case-control design. In addition, the incidence of MOTT and TB according to HIV status, the effect of HIV on clinical outcome, and the interactions between HIV and occupational lung disease will be investigated by retrospective cohort analysis of men HIV tested for reasons other than mycobacterial disease.

Propsectively, a cohort study will be initiated consisting of miners with newly diagnosed MOTT disease in order to investigate the effect of HIV infection on outcome, and the relationship between CD4+ lymphocyte count and outcome in the HIV positive men. In addition, environmental sampling for mycobacteria will be linked to a matched pair case-control study of miners with MOTT disease to investigate the extent and significance of occupational exposure to MOTT organisms.

This project started in October 1996 for three years.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Conventional and molecular epidemiology of tuberculosis in a community of gold miners in South Africa

LSHTM STAFF: Peter Godfrey-Faussett (ITD)

COLLABORATORS: Goldfields West Hospital, Westonaria; National Centre for Occupational Health, Johannesburg; University of Zambia Medical School

FUNDING: CEC, Beit Trust, Medical Bureau for Occupational Diseases.

SUMMARY:

Prospective community based study of TB in a group of four South African goldmines. All isolates since September 1994 are being typed for molecular epidemiology. Detailed mapping and interviews with TB cases within the mines since January 1995. High transmission rates despite a control programme that meets the WHO targets. Analysis for cohort diagnosed in 1995 almost complete. We are continuing to follow up 1996 isolates. Work on the extended analysis is still continuing.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Effect of TB on progression of HIV disease

LSHTM STAFF: Alwyn Mwinga, Maria Hosp, Peter Godfrey-Faussett, John Porter, Maria Quigley (ITD)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health; Innsbruck University; Hvidovre Hospital, Copenhagen .

FUNDING: WHO, DFID, Innsbruck University, Hvidore Hospital.

SUMMARY:

In vitro, ingestion of *Mycobacterium tuberculosis* by macrophages leads to increased expression of HIV. Retrospective, case-control studies suggest that among patients matched for HIV disease state by CD4 count, those who develop tuberculosis progress more quickly than those who do not.

Progression markers for HIV disease (CD4 lymphocyte count, beta-2-microglobulin and neopterin) have been measured regularly in the cohort of 1053 individuals in the preventive therapy study. Analysis will show whether preventing TB in the two active arms of the trial was also effective in reducing the rate of decline of immunity, as measured by these markers. A case-control study of those individuals who developed TB compared to progression matched controls will also be undertaken.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Tuberculin skin test conversion and reversion among an HIV positive cohort given preventive therapy.

LSHTM STAFF: Alwyn Mwinga, Maria Hosp, Peter Godfrey-Faussett, John Porter, Maria Quigley (ITD)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health

FUNDING: WHO, DFID

SUMMARY:

In regions where both HIV and *Mycobacterium tuberculosis* are highly prevalent, TB may arise as a reactivation of latent infection, as a new infection or as a reinfection. Preventive therapy is effective in reducing the first of these for a prolonged period and may also reduce the latter two mechanisms during the period of therapy. Tuberculin skin tests are used to determine who has already been infected with *Mycobacterium tuberculosis*, but with HIV related immunosuppression, the test becomes increasingly insensitive.

By analysing sequential tuberculin tests performed in the cohort and relating them to the degree of immunosuppression and the treatment received, we are trying to determine the rate of new infection and the mechanisms by which preventive therapy works.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Counselling, screening, recruitment and compliance - implications for implementation of preventive therapy

LSHTM STAFF: Alwyn Mwinga, Maria Hosp, Peter Godfrey-Faussett, John Porter, Rachel Baggaley, Maria Quigley (ITD), Susie Foster (PHP)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health; Kara Counselling and Training Trust

FUNDING: WHO, DFID

SUMMARY:

Preventive therapy for TB is the first medical intervention that has been proven to benefit those infected with HIV in Africa. However, a feasibility study examining the potential for a TB preventive therapy programme in Uganda demonstrated that so many people were lost during the process that the programme was unlikely to be cost-effective. By analysing data on the value of different screening tools to exclude active TB; different approaches to counselling and recruitment; risk factors for non-compliance; and factors associated with efficacy of preventive therapy we aim to develop guidelines for more targeted use of treatment.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Diagnosis and outcome of children suspected to have TB

LSHTM STAFF: Peter Godfrey-Faussett (ITD)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health

FUNDING: WHO, CEC

SUMMARY:

Tuberculosis is usually diagnosed in children by using a clinical scoring system since microbiological proof is rarely obtained. Unfortunately, many of the features suggestive of TB are also common findings in children infected with HIV with complications other than TB.

We have completed a prospective cohort study of 202 children with "suspected" TB. Data cleaning and analysis has started. The aim is to define which signs, symptoms and laboratory tests at admission would have allowed us to predict which children had TB, which would benefit from treatment and which would die. We plan to use the data to design a further study testing better diagnostic approaches to the problem.

LSHTM TUBERCULOSIS – RELATED RESEARCH

TITLE: Evaluation of the role of new nucleic acid amplification tests in the routine diagnosis of tuberculosis in developing countries with a high prevalence of HIV infection

LSHTM STAFF: John Porter, Peter Godfrey-Faussett, Ruth McNerney, Vincent Tihon (ITD) Susie Foster (PHP)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health; St. Francis Hospital Katete

FUNDING: DFID, WHO, Beit Trust

SUMMARY:

HIV-related tuberculosis is associated with fewer positive sputum microscopy results. For instance, in Lusaka in 1995, only 46% of pulmonary tuberculosis cases had a positive sputum microscopy result. The huge increase in the number of patients over the past decade has caused logistical difficulties in the diagnostic laboratory. More sensitive rapid diagnostic tools could lead to more accurate diagnosis and treatment and might improve the efficiently of the TB control programme.

An assessment of the feasibility, accuracy and cost-efficacy of nucleic acid amplification for the diagnosis of pulmonary tuberculosis is being undertaken. The study is being conducted in both a regional centre setting and in a rural district hospital.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: DNA Fingerprinting using IS6110 in Zambia.

LSHTM STAFF: Peter Godfrey-Faussett, Maria Quigley (ITD)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health; National Institute for Environmental Protection, Bilthoven

FUNDING: CEC, Beit Trust

SUMMARY:

Analysis and manuscript preparation are ongoing for studies on Zambian *Mycobacterium tuberculosis* strains. Genetic diversity; multiple infections in a single host; mechanisms of recurrent disease; frequency of laboratory contamination in a high throughput, low resource laboratory. Preparations have been made for a community based molecular epidemiology study to tie in with the Katete PCR study (see project on new nucleic acid amplification tests).

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Studies with the Zambian National TB Reference Laboratory
LSHTM STAFF: Peter Godfrey-Faussett (ITD)
COLLABORATORS: Chest Diseases Laboratory, Zambian Ministry of Health; University of Zambia Medical School; Mycobacterium Reference Unit, Dulwich PHLS
FUNDING: CEC, WHO, DFID, Beit Trust

SUMMARY:

As part of the collaboration between the LSHTM and the Zambian Tuberculosis Control Programme (the ZAMBART project), we have been supporting the national reference laboratory with training (both in London and Lusaka), equipment and reagents. Several projects have been undertaken within the laboratory:

1. Quality assurance of microscopy

Around 40% of positive cultures of *Mycobacterium tuberculosis* at the chest diseases laboratory come from samples that were initially reported as being negative on microscopy. A computer programme has been designed to produce a sample of slides to be re-microscoped to determine whether these results are correct or whether the huge turnover of slides (>35,000 per year) has led to under-reporting of positive slides. Some clerical and technical errors have been uncovered but they account for less than 15% of microscopy negative yet culture positive samples.

2. Improving routine data processing

A data processing system has been installed which runs in parallel to the paper results at present. Descriptive statistics of the laboratory's work are now easily available and have been used to plan future developments for the Lusaka microscopy services. Microscopy results are retrieved for the chest clinic for those patients in whom the paper result has been mis-filed or lost

3. National survey of drug resistance

A nationally representative sample of patients has been identified using proportional to population sampling from each district. The pilot districts have returned their specimens, which show low rates of resistance to anti-tuberculous drugs. The remaining districts are currently collecting their specimens.

4. Survey of prevalence of mycobacterium bovis.

Retrospective review of more than 5000 isolates of mycobacterium tuberculosis complex strains shows that although some of them grew better on lowenstein jensen medium containing pyruvate than medium with glycerol, none of these were identified as mycobacterium bovis as they were all resistant to thiophen carboxylic acid hydrazide. 98% of these isolates arose from Lusaka and 95% of them came from sputum samples. Since *Mycobacterium bovis* is more likely to be found in rural locations with more cattle and from extrapulmonary specimens as well as pulmonary ones, a study is now being designed to collect a more representative sample of specimens and to identify them both by TCH susceptibility and by molecular methods.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Tuberculosis control in an urban area with a high prevalence of both HIV and *Mycobacterium tuberculosis*

LSHTM STAFF: John Porter, Vincent Tihon, Peter Godfrey-Faussett, Maria Quigley (ITD), Jessica Ogden, (PHP)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; Chest Diseases Laboratory, Zambian Ministry of Health; Lusaka Urban District Health Management Team; Royal Tropical Institute, Amsterdam; University of Alabama

FUNDING: EC

SUMMARY:

This project started in October 1997. The aim is to develop and use action research approaches to improve tuberculosis control in the city of Lusaka. The outputs should be not only better control but a better understanding of the qualitative methodologies involved and which are most appropriate to which problems. Several smaller studies with overlapping objectives have been undertaken or designed by postgraduate students attached to the ZAMBART project:

1. Focus group discussions with adherent and non-adherent TB patients
These have demonstrated that non-adherence often follows logistic problems at the treatment centres, which are over-burdened by the huge case load (more than 10,000 cases in Lusaka in 1996) and poor communication and transport infrastructure. The recruitment for these groups also highlighted some of the inaccuracies of routine statistics. Several of those listed by the health centre as non-adherent had in fact died and others had clearly received treatment, which had not been recorded at the health centre.

2. Qualitative study of TB patients who fail to start treatment
Another problem that has been identified is that there are patients who bring sputum samples for examination and are found to have positive microscopy but do not return to start treatment. Since there are minimal resources available for tracing such individuals, they are often not followed up. A study has been developed to explore the size of this problem and to identify why such patients do not follow through their initial plan to be treated in the government health sector and to whom they turn instead.

3. Availability of TB drugs over the counter in pharmacies in Lusaka.
Although they are supposed to be available only on prescription, this study has shown that all the common anti-tuberculous drugs can be bought over the counter in large pharmacies in Lusaka.

4. Delays in diagnosis and treatment, a comparison between Lusaka and Monze.
In order to minimise transmission of tuberculosis, the delay from the time the patient develops symptoms until the time that they start treatment should be as short as possible. This delay is made up of the patient's delay in seeking treatment and the health service's delay in making a diagnosis and starting treatment. This study has collected data to compare these "delays" in an urban and a rural population. Analysis is ongoing.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: The economic impact and burden on patients prior to a diagnosis of tuberculosis in Lusaka

LSHTM STAFF: Peter Godfrey-Faussett, (ITD) Susie Foster (PHP)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital, Lusaka; McMaster University, Canada

FUNDING: DFID; Beit Trust; McMaster University; Rotary Club; Ontario Ministry of Health; Ernst and Young Chartered Accountants (Canada)

SUMMARY:

This study investigates the pre-diagnosis economic impact, burden and barriers to care-seeking for tuberculosis patients in urban sub-Saharan Africa. 202 patients with new pulmonary tuberculosis were interviewed in depth and details of all patient and care-giver expenditures and lost incomes recorded.

Patients were unwell for an average of two months prior to starting treatment and during that time, spent an average of 157% of their mean monthly income seeking diagnosis and treatment.

The study has also generated considerable qualitative data on care-seeking behaviour in Lusaka and this is being analysed further.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Impact of HIV on community infection ratio of tuberculosis in Lusaka, Zambia.

LSHTM STAFF: Peter Godfrey-Faussett, Maria Quigley (ITD)

COLLABORATORS: University of Zambia Medical School; University Teaching Hospital. Lusaka

FUNDING: Beit Trust; University of Zambia

SUMMARY:

HIV has caused a huge rise in the incidence of tuberculosis in many countries in central and Southern Africa. HIV is associated with a much greater chance of progressing from latent infection to active disease. However, patients with HIV-related tuberculosis have less tubercle bacilli in their sputum and are less infectious than those without HIV. In order to assess the impact of HIV on the risk of infection in the community, we recruited 100 patients with smear positive pulmonary tuberculosis who consented to undergo HIV testing. Their households and 200 neighbourhood matched control households were visited. A questionnaire was administered covering known risk factors for TB transmission and all children between the age of 2 and 12 were skin tested using rt-23 tuberculin.

The results confirm that HIV negative tuberculosis patients are more infectious than those with HIV co-infection. The difference can be partly explained by the larger number of bacilli in their sputum. Furthermore, children living in the homes of HIV positive smear positive tuberculosis patients are no more likely to be tuberculin skin test positive than community matched controls of the same age.

HIV positive patients seem to contribute little to the infectivity of the environment in Lusaka. They do however pose a serious threat to tuberculosis control programmes, which are overwhelmed by the numbers of patients now needing treatment.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Seeking Treatment for AIDS-related illness in Lusaka: Diarrhoea, TB and STD

LSHTM STAFF: Jessica Ogden (PHP), John Porter, Sarah Lewin, Vincent Tihon (ITD)

COLLABORATORS: S.Wallman (UCL), P.Kelly (St. Barts), G.Bond and P.Ndubani (University of Zambia)

FUNDING: Being sought

SUMMARY:

Recent ethnographic and epidemiological data will be drawn upon in order to understand the effects of having an AIDS-related illness on treatment-seeking for these common infections.

New fieldwork pursuing important emerging themes will be conducted by local field staff in collaboration with the London-based team.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Genetics of host susceptibility to tuberculosis in Zambia

LSHTM STAFF: Catherine Mee, Neil Stoker (ITD)

COLLABORATORS: Adrian Wolfson, MRC Cambridge

FUNDING: DFID, MRC

SUMMARY

Catherine Mee from the ZAMBART Project based at the University Teaching Hospital in Lusaka, Zambia was funded to study for an MSc in Applied Molecular Biology of Infectious Diseases by at LSHTM by DFID. Her research project focused on the genetics of host susceptibility to tuberculosis in Zambia. The work is currently still ongoing for another year.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Epidemiological investigations of the human immune response to *Mycobacterium tuberculosis*.

LSHTM STAFF: Alison Elliott, Wendy Hodsdon, Tamara Hurst, Greg Bancroft, Hazel Dockrell, David Mabey, Richard Hayes, Maria Quigley, Jimmy Whitworth (ITD)

COLLABORATORS: P Kaleebu, B Biryahwaho, Neil French, Uganda Virus Research Institute.
A Okwera, R Mugerwa, J Ellner, Makerere University/ Case Western Reserve University Research Collaboration, Mulago Hospital.
Terry Potter, Steve Dow, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado.

FUNDING: The Wellcome Trust

SUMMARY:

The project is being conducted in Uganda at the Uganda Virus Research Institute and at Mulago Hospital. It has three components:

Study 1 is designed to examine the hypothesis that "type 1" cytokines are essential in protective immunity to *Mycobacterium tuberculosis* and that suppression of "type 1" or a switch to a "type 2" profile is deleterious among HIV positive subjects. Participants in an existing cohort (the Entebbe Pneumococcal Vaccine Study) are being enrolled, cytokine responses to mycobacterial antigens are being examined, and the relationship between cytokine responses and progression to active tuberculosis is being determined prospectively.

Study 2 is designed to examine the effect of a glucocorticoid, prednisolone, given as an adjunct to anti-tuberculous therapy, on the progression of HIV disease in HIV positive patients with tuberculous pleurisy. This study will be conducted at Mulago Hospital, Kampala.

Study 3 is designed to examine the effect of helminth infestation on the human immune response to *Mycobacterium tuberculosis*. This study is planned to take place in phase three of the project, but preliminary work is being conducted by examination of stool samples from participants in study 1.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Environmental and genetic risk factors for tuberculosis disease

LSHTM STAFF: Steve Bennett (ITD)

COLLABORATORS: Christian Leinhardt, Melanie Newport, Adrian Hill, Peter Aaby, Oumou Sow

FUNDING: MRC and possibly EU

SUMMARY

Incident cases of TB will be recruited at a clinic. A within-household control will be recruited and also a neighbourhood control. The former will be compared with cases for a range of host (genetic and non-genetic) factors and the latter will be compared for environmental factors.

DNA will be taken from cases, and their parents or siblings, and association of disease with candidate genes will be tested by the transmission disequilibrium test.

The study will start in early 1998 in The Gambia and Guinea Bissau, and later in Guinea Conakry if EU funding is obtained.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Genetic linkage to immune responses in tuberculosis in The Gambia

LSHTM STAFF: Steve Bennett, Shabbar Jaffar, Helen Weiss (ITD)

COLLABORATORS: Annette Jepson, Winston Banya, Hilton Whittle

FUNDING: The Wellcome Trust

SUMMARY

Over two hundred adult twin pairs were recruited as part of a malaria study. A further sample has been taken to measure immune responses to TB antigens. By comparing MZ and DZ twins, the heritability of these responses can be measured.

The DZ twins are then each typed at 300 microsatellite markers along the genome. For those responses with a high heritability, multipoint affected sibling pair methods are used to demonstrate the level of linkage of the response to any point on the genome. If linkage is found to an area, more detailed studies will be carried out to locate the relevant gene.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A study of policy development and implementation of the National Tuberculosis Programme of India

LSHTM STAFF: Gill Walt (PHP), Thelma Narayan, Community Health Cell, Bangalore (PhD Student)

COLLABORATORS:

1. Cebemo, The Netherlands;
2. Misereor, Germany;
3. DFID Programme on Tuberculosis, LSHTM;
4. Memisa, The Netherlands

FUNDING:

SUMMARY:

This study aims to understand underlying explanations for the perceived gap between policy expectations and achievements of the National Tuberculosis Control Programme of India (NTP). A policy approach is being used as the analytical framework.

The study has two parts:

a) A National level, historical review of the interplay between broad contextual factors, the policy process of development and implementation of policy, and the actors who have been central to that process.

b) A study of process and implementation at State (Karnataka), district (Mysore) and sub-district level. It will focus on the point of contact between the services of the NTP and the patient. To understand the interaction, qualitative methods of research will be used with TB patients, health workers and primary health centre Medical Officers. Comparison will be made of process and outcome in two taluks (sub-district level), one with public sector services and another in which a voluntary organisation has taken over responsibility for the NTP from Government. Interviews will be held in both taluks with elected representatives regarding the functioning of general health services with which the NTP is integrated.

At District, State and National level, research methods include interviews with a wide range of people and collection of data concerning the NTP, including perusal of records, resource flows, documents, evaluation reports and other literature.

Fieldwork in India started at the end of September 1995, for one year. Links are being maintained with National and State government institutions and non-government organisations involved in the field of tuberculosis.

There has been a smaller focus on the Revised NTP, the current phase of the policy process and on the role of international players over the years.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Operations research to assess needs and perspectives of TB patients and providers of tuberculosis care in Nehru Nagar and Moti Nagar chest clinics areas of Delhi.

LSHTM STAFF: Jessica Ogden (PHP), John Porter, Sarah Lewin (ITD).

COLLABORATORS: Varinder Singh, R.C. Jain, Lala Ram Swarup Institute for Tuberculosis and Allied Diseases, Delhi; Mukund Uplekar & Sheela Rangan, Foundation for Research in Community Health, Bombay.

FUNDING: DFID Health and Population Office, Delhi

SUMMARY:

Operations research was conducted to contribute to ongoing efforts to improve the accessibility and acceptability of tuberculosis services in India under the Revised National Tuberculosis Programme. The study employed a range of qualitative and quantitative research methods in order to explore the social, cultural and economic factors which influence treatment-seeking for tuberculosis symptoms among the slum populations in these two chest clinic areas. The study also explored the perceptions, activities and experiences of local public and private sector providers in treating tuberculosis. In particular the implementation of DOTS was observed and the thoughts and feelings of current patients and DOTS providers about the programme were recorded.

Key policy-relevant findings:

1. The most vulnerable groups are likely to be recent migrants into the poorer slum areas in Delhi. These people are likely to lack localised networks of social support which facilitate obtaining access to key material and non-material resources, and which are influential in the ability of patients to adhere to demanding TB treatment regimens.
2. The private, non-formally qualified practitioners working in the slum areas are the key treatment source for all symptoms, particularly early on in the course of an illness. They are an important link in the chain of health-shopping, and are a potential resource both in terms of referral into the RNTP and as neighbourhood DOTS supervisors.
3. Positive aspects of the Revised programme include
    - regular, uninterrupted supply of drugs
    - blister packs
    - recording and reporting systems
4. Negative aspects include
    - cure rate targets are precipitating pre-selection of patients for RNTP and many of the most vulnerable patients are being selected-out of the revised programme
    - inflexibility of DOT component of the programme means that many patients are not able to continue with treatment once they are well enough to go back to work or school
    - poor infrastructure of DOTS clinics reflect poor linkages with the general health services and local NGOs.
    - Lack of provision for the treatment of side-effects and co-infections

State and District-level dissemination workshops are planned for May 1998.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Operations research for improving the efficiency of RNTP in Medak District, Andhra Pradesh

LSHTM STAFF: John Porter (ITD), Jessica Ogden, Ruairi Brugha (PHP), Sarah Lewin (ITD)

COLLABORATORS: G.N.V. Ramana, B.B Sharma and C. Naidu, Administrative Staff College of India, Hyderabad; Mukund Uplekar and Sheela Rangan, Foundation for Research in Community Health, Bombay.

FUNDING: DFID Health and Population Office, Delhi

SUMMARY:

The overall objective of the project was to develop specific guidelines for improvements to the Revised National Tuberculosis Programme in Medak and to provide a model for the future up-scaling of the revised strategy in the State of Andhra Pradesh. The project had both "supply-" and "demand-" side dimensions: that is the perceptions, expectations and behaviours of both providers of tuberculosis treatment, as well as patients and communities. A range of qualitative and quantitative methods were employed including a household survey to identify chest symptomatics, current and past TB patient and provider interviews, facility assessments, non-participant observations and a series of community focus group discussions in rural (tribal and non-tribal) villages and urban areas (both high and low case clustering). A community mapping exercise was also carried out to identify the location of private sector providers in the district.

Key findings:

- Owing to deficiencies in public provision of primary health care, private qualified and non-qualified providers are an important source of treatment for local people, but their TB treatment practices are not in accordance with the revised programme.
- About 1/5 of the current TB patients waited more than three months from the onset of symptoms to the first consultation of any provider
- X-Ray continues to be the most important diagnostic method for both patients and providers
- In the implementation of the RNTP one of the key difficulties is obtaining sputum and repeat sputum samples for testing. Due to lack of necessary infrastructure and equipment fewer than half of the participating primary health institutions were performing sputum tests.
- DOTS in the rural areas is operationally difficult due to the vast distances involved and the lack of geographical access due to poor roads and poor public transport.
  - ⇒ Defaulter retrieval should occur within 24 hours of default, but in practice field staff make take a minimum of 3 - 4 weeks before following patients up
  - ⇒ The concept of neighbourhood DOTS was discussed in the communities and they were on the whole enthusiastic, mainly identifying local politicians as potential DOTS supervisors.
- The main gender differences were in relation to duration of symptoms prior to diagnosis (with women waiting sometimes up to three times as long) and in terms of the stigma associated with TB (with women being more strongly effected).

State and District-level dissemination workshops are planned for May 1998.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: State of the art reviews on operational research in tuberculosis, India

LSHTM STAFF: John Porter (ITD), Jessica Ogden, David Nyheim, Anthony Zwi, Ruairi Brugha (PHP)

COLLABORATORS: M.Uplekar and S.Rangan, Foundation for Research in Community Health, Bombay

FUNDING: DFID Health and Population Office, Delhi

SUMMARY:

To review and critically assess existing knowledge of the global and Indian experience of tuberculosis and the individual, community, professional, national and international responses to it in order to identify mechanisms for improving TB control in India, and to highlight key areas to be covered by DFID-funded operational research.

The report was completed in 1997. A final version is to be published in 1998.

LSHTM TUBERCULOSIS – RELATED RESEARCH

TITLE: Principles of the DFID TB Programme policy working group – India

LSHTM STAFF: Jessica Ogden, David Nyheim, Anthony Zwi, Ruairi Brugha (PHP) John Porter (ITD)

FUNDING:

SUMMARY:

In the course of working together on the State of the Art Literature Reviews the group has identified a number of key themes which are central to developing TB control policy in a meaningful and fruitful way.

1. Gender:
Until recently the importance of gender in relation to tuberculosis has been under-appreciated. **Sex** and **gender** are often wrongly conflated, resulting in a decontextualised understanding of the available epidemiological evidence.

Key issues in TB which are related to / influenced by gender, include:

- rates of, and vulnerability to, infection;
- access to and use of health care providers;
- differences in the effects of social stigma; and
- structural factors (e.g. poverty) which affect people differently according to gender.

2. Public Health Ethics:
Several key issues are involved here, notably the difficulty of finding the appropriate balance between the rights of individuals and communities to attain or maintain dignity and autonomy in the control of their health and the need to protect communities from outbreaks and pandemics of dangerous infectious diseases and the development of drug-resistant organisms.

3. Human Rights:
These issues relate to those included under Public Health Ethics and gender; but are located within the context of global, national and local structures which promote inequality and inequity in obtaining access to those resources fundamental to promoting and maintaining health, including education, employment, housing, sanitation, food, and political power.

4. Political economy and health sector reform:
All three of the above themes rest within broader processes such as the globalisation of the world economy, the increase in social marginalisation, the decline in the role of the state, as well as within the context of health sector changes notably privatisation, other aspects of health sector reform, and decentralisation.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: TB and Leprosy control in Orissa, India

LSHTM STAFF: John Porter (ITD)

COLLABORATORS: LEPRA, India

FUNDING:

SUMMARY:

There are plans to work with the non-governmental organisation LEPRA on the process involved in integrating TB and leprosy services. Currently LEPRA has a pilot study in a tribal region of Orissa state in India, which involves the use of leprosy workers for TB control activities. The research will include work on how to ensure that 'hard to reach TB patients' are included in the TB control services.

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LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Infectious Disease Policy Group (Health Policy Unit)

LSHTM STAFF: J.Porter (ITD), J.Ogden, Anthony Zwi, Ruairi Brugha, G.Walt, H.Goodman, L.Lush, P. Lloyd-Sherlock, D.Sethi, C.Watts, S.Foster, K.Lee-Gilmore, T.Narayan, S.Mayhew, M.Bhatia, G.Howe (PHP)

FUNDING: This is a School-based "interest" group

SUMMARY:

Some of the dimensions of Infectious Disease Policy being addressed by this group:

- Multi-disciplinary (IDP means couching our epidemiological, biomedical, basic science and demographic knowledge within a more considered understanding of the structural determinants of health and disease - political economy, social structures, health systems, ethics and human rights)
- Bringing the people back in (otherwise known as "upstream/downstream" issues; participatory approaches)
- Looking at processes of health sector reform and decentralisation. It is vital that we begin to make horizontal (or "diagonal") linkages and to find ways of describing what is going on and what factors are influencing people's health.
- Raising a challenge to the prevalent view that "policy" is just about "programmes" (with the focus on disease specificity, verticality, short-termism emphasising products not process).

Broad areas for focus-

1. Clarifying what we mean by Infectious Disease Policy
2. Identifying key research questions
3. Identifying the role for LSHTM in addressing them
4. Thinking about methodological approaches
5. Engaging with others in the School with related concerns
6. Engaging with our southern research partners in this process

Some preliminary questions:

1. Under what circumstances are communities/marginalised groups able to exercise power to influence policy/providers?
2. What are the key (social) indicators?
3. How do structural factors affect the diseases people get and their access to health care?
4. How do structural factors affect people's perceptions of health and well-being?
5. On balance are some infectious diseases more "important" than others?
6. What role should private providers have in any IDP?
7. How appropriate are discourses of *eradication* and *elimination* of disease to IDP?
8. How do northern perceptions and the interventions and policies they generate influence the south?
9. Macro-level factors leading to decline in public health standards
10. Transition and conflict - contributions to communicable disease transmission; barriers to control

Some organising themes:

- Current debates on a direction for public health in the 21st century, indicate the need to move away from the belief that the key to health is the elimination disease to a concept of public health based on the creation production of health: it is a shift from reaction to pro-action, and it supports the view that "health will be better promoted by the affirmation of the skills and concepts that advance it, than by emphasis on the factors that impede it".
- These debates have arisen from the rapid societal changes brought about by globalization, the increasing inequity both within and between countries, and a perception that public health is failing to stay abreast of the rapid changes affecting the health of the world's populations.

Developing the concept of Infectious Disease Policy will encourage a change in focus from a purely biomedical approach to one which includes political economy, gender, equity, ethics and human rights. It will also help to change the research agenda to include a wider multidisciplinary platform and the creation of alliances between researchers working in disciplines and sectors within and outside the health professions.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Role of antigen-specific CD8+ T cells in immunity to tuberculosis

LSHTM STAFF: Hazel Dockrell (ITD)

COLLABORATORS: Pauline Lukey, Glaxo Wellcome Research & Development
Graham Bothamley, Homerton Hospital, London

FUNDING: MRC Industrial Collaborative Studentship (autumn 1997)

SUMMARY:

Controversy exists as to the function of major histocompatability complex (MHC) class I restricted $CD8^+$ T cells in a protective immune response to mycobacterial infection in man. Our hypothesis is that $CD8^+$ T cells play a role in protective immunity by producing interferon-γ (IFN-γ) and lysing autologous infected macrophages. The aim of this project is to determine the role of $CD8^+$ T cells in immunity tuberculosis. The specific aims are to assess whether activation of $CD8^+$ T cells is higher in healthy BCG vaccinated donors than in patients with tuberculosis; can such mycobacteria-specific $CD8^+$ T cells function as cytokine secreting and/or cytotoxic effector cells; and what is the MHC restriction of these mycobacteria specific $CD8^+$ T cells.

Techniques to be used will include lymphocyte proliferation assays to assess antigen-specific T cell proliferation, limiting dilution analysis to determine precursor cell frequency, analysis of T cell activation using FACScan, cytokine production by ELISA and intracellular cytokine staining, and the standard chromium release cytotoxicity assay. A range of strategies will be employed to induce $CD8^+$ T cell activation, including the use of live mycobacteria (initially *M. bovis* BCG and later *M. tuberculosis*), presentation of antigens on latex particles, and the use of recombinant vaccinia expressing secretory antigens from *M. tuberculosis*. Peripheral blood mononuclear cells from normal donors with/without BCG vaccination, and from patients with tuberculosis will be used, $CD8^+$ T cells will then be purified using immunomagnetic bead separation.

Preliminary data from the laboratory has demonstrated a capacity for $CD8^+$ T cells to recognise and become activated (i.e. upregulate the IL-2 receptor CD25) in response to mycobacterial antigens. We have now shown by intracellular cytokine staining and flow cytometry that these activated $CD8^+$ T cells produce IFN-γ. In addition the observed activation and cytokine production was greatest in response to live *M. bovis* BCG as opposed to the soluble antigen PPD. The next step is to stain for other cytokines such as IL-2, IL-4, and TNF-α, to identify whether the activated $CD8^+$ T cells are type 1 or type 2 cytokine producers. $CD8^+$ T cells from healthy BCG vaccinated donors and tuberculosis patients will be compared to see if there is any correlation between cytokine production and immunity to disease.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A randomised controlled trial of a second dose of BCG vaccination against tuberculosis

LSHTM STAFF: Laura Rodgrigues (ITD)

COLLABORATORS: Professor Mauricio Barreto, Universidade Federal da Bahia. Brazil;
Dr Miguel Ayub Ajjar, Director, TB Control programme, Brazil

FUNDING: DFID and Brazilian Government

SUMMARY:

The overall aims of the existing project are:

1. To estimate the efficacy of BCG vaccine (given to school children in a population with a high coverage of neonatal BCG) against tuberculosis and
2. To provide an estimate to the cost of vaccination and of the cost of vaccinating enough children to prevent one case.

Neonatal BCG vaccination is recommended by WHO in countries with a high incidence of tuberculosis, and is believed to be the most widespread vaccine in the third world. There is evidence that BCG protection wanes with time in most settings.

WHO has pronounced recently against giving a second dose of BCG vaccine, arguing that there is no evidence that a second dose would add protection, and that scarce resources should be used for case-finding and treatment. This recommendation is controversial: many countries offer a second dose of BCG vaccination, usually at school, and do not appear to plan to stop in spite of WHO recommendation. Brazil has recently introduced a second dose.

Methods

600 schools were randomized (within 6 strata of size and socio-economic conditions of the area) into vaccination or no vaccination. 75,000 children received vaccination; another 75,000 children did not receive vaccination and were examined for a previous BCG scar and gave identifying information. Cases diagnosed through the tuberculosis control programme are identified and their case notes reviewed; confirmed cases are linked to the database of school children in the study.

Additional related work includes an immunological study looking at interferon production before vaccination according to atypical mycobacteria sensitivity.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: TNF-α and TGF-β production by human mononuclear phagocyte in response to infection by mycobacteria.

LSHTM STAFF: Hazel Dockrell (ITD)

COLLABORATORS: Pauline Lukey, Glaxo Research and Development, UK;
Graham Bothamley, Homerton Hospital, London;
Ronnie Anderson, Department of Immunology, University of Pretoria, South Africa.

FUNDING: MRC (application submitted)

SUMMARY:

TNF-α and TGF-β are important cytokines produced by human macrophages in response to various stimuli, including infection by mycobacteria. These cytokines have mutually antagonistic effects and their relative production may play a role in determining the immunological outcome of macrophage ingestion of *Mycobacterium tuberculosis*. TNF-α is thought to be required for an effective immune response and mycobacteriostasis, whilst TGF-β is largely immuno-suppressive and enhances the growth of intracellular mycobacteria. Thus, phagocytosis of virulent mycobacteria may result in relatively less TNF-α and more TGF-β than ingestion of dead mycobacteria or a less virulent strain. Furthermore, cytokine production may also be stimulated in bystander non-infected macrophages, by soluble products of mycobacteria or infected macrophages.

The aim of this study is to use ELISA, bioassays and flow cytometry to correlate infection of macrophages by mycobacteria, with production of these cytokines. Dual parameter flow cytometry will be used to correlate phagocytosis (mycobacteria will be fluoresceinated) and cytokine production (detected using phycoerythrin), allowing analysis of bystander effects. Both intracellular and surface-associated cytokines should be distinguishable by comparing labelling before and after permeabilisation of the macrophages. Mononuclear phagocytes from normal individuals will be used to standardise the methodology. Subsequent studies will involve mononuclear phagocytes from the circulation and tuberculous effusion of patients with tuberculosis. This study should provide insight into the ability of virulent mycobacteria to influence the host immune response by modulating patterns of cytokine production by the infected host macrophages.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Role of CD8+ T cells in immunity to *M. tuberculosis*

LSHTM STAFF: Joanne Turner, Hazel Dockrell (ITD)

COLLABORATORS: Dr. G. Scott (University College, Hospital, London); Professor K. McAdam, Dr. H. Whittle (MRC Laboratories, The Gambia).

FUNDING: MRC Studentship (to October 1996); Gordon Smith Travelling Fellowship.

SUMMARY:

Previous work has established a role for the CD4+ T cell in protection against infection with *M. tuberculosis*. However, antigen delivery in soluble form, such as PPD, will preferentially bias antigen presentation to the MHC Class II pathway, and so may select against the study of CD8+ T cells. To identify whether there is a role for the CD8+ T cell in immunity to *M. tuberculosis*, we have stimulated peripheral blood mononuclear cells from normal BCG vaccinated donors or from patients with tuberculosis with live *M. bovis* BCG. Using flow cytometry, we have shown that *in vitro* live *M. bovis* BCG activates significantly more CD8+ T cells than PPD, a soluble antigen. Live *M. bovis* BCG also induced greater CD8+ activation than an inactivated, non-viable suspension of *M. bovis* BCG. Using a chromium release cytotoxicity assay, both *M. bovis* BCG and PPD were shown to induce antigen specific cytotoxic T cells, and cell suspensions enriched for CD8+ T cells were also cytotoxic. Further studies are comparing CD8+ T cell activation and cytotoxicity in patients with tuberculosis at diagnosis and at various time points during therapy.

This project has finished and a PhD has been awarded.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Comparison of immune responses to live and dead mycobacteria

LSHTM STAFF: Emilia Valadas (ITD), Hazel Dockrell (ITD)

COLLABORATORS: Graham Bothamley, Homerton Hospital, London;
Jeanette Meadway, Newham General Hospital, London.

FUNDING: EC Praxis; Gulbenkhian Foundation, Lisbon

SUMMARY:

Previous work has demonstrated that live *M. bovis* BCG can activate more CD8+ T cells than a non-viable suspension of the same bacteria. Such differences in immune responses to live and dead bacteria could result from the ability of live mycobacteria to change their location within the cell, to modify the antigen processing and presentation pathways within the cell, to produce greater quantities of immunodominant secreted proteins – or merely to multiply within the host macrophage thereby increasing the bacterial load.

M. bovis BCG preparations have been collected following different lengths of culture and their viability checked using staining techniques and counts of colony formation. The ability of these preparations to induce T cell and macrophage responses is then tested *in vitro*. Preliminary results indicate that both live and dead bacteria can induce equivalent lymphocyte proliferation. However, secretion of TNFα, a cytokine which may be important in macrophage activation, is often higher in response to dead bacteria. ELISA assays and RT-PCR techniques are being used to analyse a wider panel of cytokines, including TNFα, IFN-γ, IL-4 and IL-10.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Identification of protective immune responses to pathogenic mycobacteria

LSHTM STAFF: Hazel Dockrell (ITD)

COLLABORATORS: Dr. D. Kumararatne, University of Birmingham;
Professor F. Ventura, CMDT, Lisbon;
Dr. T. Corrah, MRC Laboratories, The Gambia;
Professor F. Vega Lopez, IMSS, Mexico City;
Professor A. Hill, University of Oxford.

FUNDING: EU

SUMMARY:

The objectives of this project are to evaluate the role of T cell-associated lytic mechanisms in the killing of intracellular mycobacteria and the contribution of these effector pathways to immunity against tuberculosis and leprosy.

The specific aims are to:

1. Evaluate whether antigen-specific CD4+ and CD8+ T cell-mediated cytotoxicity reduces the survival of intracellular mycobacteria within target macrophages, and the relative contribution of cytolytic effector mechanisms, such as Fas, perforin/granzymes and exocytosis of ATP, to this T cell-induced macrophage death.

2. Assess the role of the γδ T cell subset in cytolysis and cytokine secretion in tuberculosis with/without HIV co-infection.

3. Investigate the mechanism(s) by which extracellular ATP (eATP)-induced macrophage death (occurring via the P2z-receptor mediated pathway) reduces survival of intracellular *M. bovis* BCG, and extend these studies to other pathogenic mycobacteria.

4. Investigate the role of genetic heterogeneity of eATP-induced macrophage and intracellular mycobacterial killing in conferring resistance to mycobacterial disease, by comparing P2z receptor expression on monocyte-derived macrophages from patients with tuberculosis (with/without HIV, lepromatous leprosy, and endemic controls.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: The role of γδ T-cells in *Mycobacterium tuberculosis* and HIV infection

LSHTM STAFF: Diane Ordway, Hazel Dockrell (ITD)

COLLABORATORS: Fernando Ventura, CMDT, Lisbon

FUNDING: Praxis grant to Diane Ordway, 4 years (from Spring 1997)

SUMMARY:

HIV infection with its resultant impairment of cell mediated immune response predisposes both to the development of active tuberculosis after acquisition of mycobacterial infection and to reactivation of latent infection. Although we know that acquired resistance against tuberculosis is T cell dependent, and the importance of αβ lymphocytes is beyond question, the contribution of γδ cells needs further definition.

Although the precise function of γδ T cells is still unknown, γδ T cells have been shown to proliferate specifically in response to several bacterial antigens such as killed *M. tuberculosis* or heat shock proteins (60 and 70kDa). The level of circulating γδ T lymphocytes has also been shown to increase in peripheral blood of patients with pulmonary tuberculosis, as well as in other infections including HIV. An increased expression of the activation markers HLA-DR and CD 38 has also been described in γδT lymphocytes, which correlate inversely with the CD4+ T lymphocyte count in HIV infection, suggesting an involvement of γδ lymphocytes in disease progression. Percentages of γδ cells after culture in the presence of *M. tuberculosis* (H37Ra) have been found to be higher in tuberculin reactors and in patients with pleuritis than those with pulmonary or miliary tuberculosis, suggesting that they may contribute to mycobacterial resistance.

This study, which started in Spring 1997, seeks to determine if the activity of γδ T cells in HIV disease is secondary to the response to *M. tuberculosis* infection. The function of γδ T cells will also be assessed by measuring activation, cytokine secretion and cytotoxicity.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: The role of cytokines and apoptosis in mycobacterial killing

LSHTM STAFF: Hazel Dockrell, Sara Atkinson (ITD)

COLLABORATORS: Pauline Lukey, Glaxo Wellcome; Dr. Graham Bothamley, Homerton Hospital

FUNDING: MRC Small Project Grant (ended Oct 97) and new application submitted

SUMMARY:

The aim of this project is to determine the mechanisms by which human mononuclear phagocytes kill mycobacteria, using *M. bovis* (BCG) as a model system. In mycobacterial infection the monocyte/macrophage plays an important role in determining the overall outcome of the disease. We will study two methods by which monocytes/macrophages may overcome infection (1) by producing pro-inflammatory cytokines and thereby inducing cell activation and a subsequent cascade of immunological events and/or (2) by inducing apoptosis (programmed cell death). This project sets out to determine the relative importance of these mechanisms in bacterial killing.

PBMCs from healthy donors and patients will be obtained. Monocyte-derived macrophages(MDMs) will be cultured and infected with BCG labeled with fluorescein. The proportion of MDMs containing BCG and expressing intracellular cytokines will be assessed by FACS analysis. The level of apoptosis will be determined by FACS analysis and by the TUNEL method. The effect of these mechanisms on mycobacterial killing will be assessed. Future experiments will address the role of nitric oxide in mycobacterial killing.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Immunological correlates of protection by BCG

LSHTM STAFF: Rosemary Weir, Hazel Dockrell, Paul Fine (ITD)

COLLABORATORS: Professor J M Blackwell, University of Cambridge

FUNDING: Application submitted

SUMMARY:

Glaxo (now Evans) BCG vaccine has consistently been shown to provide strong protection against pulmonary tuberculosis in the UK, although the same vaccine has failed to provide any protection against this disease in Malawi. Differences in exposure to environmental mycobacterial and/or in genetic background between populations may account for this variation in BCG efficacy. To date, there have been no large scale studies in which the immunological responses induced by BCG vaccination in the UK have been defined and quantified. Newly established and validated whole blood assays, suitable for immunoepidemiological use, can now be used to measure the production of various cytokines, the best available indicators of immune responses to vaccination. This study will take advantage of the UK-Malawi dichotomy to identify constituent steps and correlates of the protective responses induced by BCG vaccination in the UK, by comparing the detailed immunological responses of individuals to Glaxo-Evans BCG vaccine between these two populations, as a function of their prior mycobacterial sensitivity and genetic profiles.

Protective immunity to tuberculosis is thought to involve bacterial killing by activated macrophages, induced by the secretion of macrophage-activating cytokines, and in particular the Type 1 cytokine interferon-γ (IFNγ), by antigen-specific T-cells. In this study, which will be organised through the routine school BCG vaccination programme in the UK, whole blood assays will be used to measure pre- and post-BCG vaccination immune profiles of British adolescents in terms of cytokine production induced by mycobacterial antigens. The antigen panel will include proteins from a variety of vaccine, pathogenic and environmental mycobacteria, in order to investigate the influence of prior mycobacterial exposure on naturally-acquired and vaccine-attributable responsiveness. Responses will be measured in terms of macrophage-derived cytokines (TNFα and IL10) as an indication of the innate non-specific macrophage response, and T-cell derived cytokines (IFNγ, IL5 and IL10) to assess the antigen-specific acquired immune response. The cytokine responses will be analysed in relation to polymorphisms in several candidate macrophage and T-cell response genes, in order to identify genetic determinants of natural and vaccine-associated immune responsiveness. Comparisons between these parameters in the UK population (this study), with those measured in the Malawi population (a Wellcome Trust-supported study currently underway), should reveal correlates of BCG-attributable protection against tuberculosis and other mycobacterial diseases.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: TB control in Venezuela

LSHTM STAFF: John Porter (ITD)

COLLABORATORS: CAICET, Venezuela

FUNDING: British Council

SUMMARY

Plans are being developed for a research initiative with CAICET (Centro Amazonico para la investigacion y el control de las enfermedades tropicales) in Venezuela. In the southern part of Venezuela there are small Amazonian communities with a high incidence of tuberculosis. The research, which will be both qualitative and quantitative, will look at the transmission of TB, treatment and control.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: DFID Tuberculosis Programme

LSHTM STAFF: John Porter, Ruth McNerney, Sarah Lewin, Judith Glynn (ITD), Jessica Ogden (PHP)

COLLABORATORS: Klaus Jochem, Nuffield Institute for Health, Leeds

FUNDING: DFID

SUMMARY:

The tuberculosis programme links the London School of Hygiene & Tropical Medicine with the Nuffield Institute for Health, Leeds. It is a multidisciplinary programme with research in basic science, clinical medicine, epidemiology, economics and social science. The aim of the programme is to address and help to overcome the problems faced by TB control programmes in developing countries, through applied research, particularly in countries affected by the HIV pandemic. At present, the programme is concentrating its efforts in the following countries: Malawi, Kenya, Zambia, India, Pakistan and Nepal.

Details of individual studies supported by the programme are provided under separate headings within this booklet.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Development and validation of technologies for rapid, sensitive diagnosis of tuberculosis that are appropriate for use in low-income countries and transfer of new technology via training and technical support

LSHTM STAFF: Ruth McNerney, Neil Stoker, John Porter, Zubaida Al-Suwaidi (ITD)

COLLABORATORS: Dr Stuart Wilson (Dulwich PHLS)

FUNDING: DFID

SUMMARY:

A low-cost, rapid and sensitive method for the diagnosis of tuberculosis has been developed at the School. The method utilises viruses (mycobacteriophages) that will infect and replicate in viable mycobacteria. A simple, low-cost assay was developed following our discovery that ferrous (iron II) compounds can be used to kill mycobacteriophages while not harming the bacteria or those phages replicating inside them. Phages are incubated for one hour with samples suspected of containing *M. tuberculosis* bacteria. Following treatment for a few minutes with ferrous ammonium sulphate any phage then detected are the result of replication inside a bacteria, and indicates the presence of live mycobacteria in the sample. The progeny phages are detected by the formation of plaques (clear areas) in a lawn of a fast-growing mycobacteria, a process which gives visual detection within 16 hours and that needs no specialist equipment. The assay was shown to detect *M. tuberculosis* in sputum specimens in collaboration with University College London Hospital and was used for the rapid detection of rifampicin resistance in isolates at Iskrez Hospital, Bulgaria. Further validation work on drug susceptibility testing is being undertaken at the PHLS Mycobacterium Reference Unit.

A commercial kit based on this technology is being developed by Biotec Laboratories for the diagnosis of pulmonary tuberculosis and further collaborative work is to be undertaken on the development of a kit for drug susceptibility testing.

Specialist training in molecular techniques and the new phage technology for the identification of tuberculosis has been undertaken both overseas and by numerous visitors to our laboratory in London.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Cloning and characterisation of genes involved in cell wall synthesis in *Mycobacterium tuberculosis*

LSHTM STAFF: Tanya Parish, Neil Stoker (ITD)

COLLABORATORS: Patrick Brennan, Colorado State University, Fort Collins; Stewart Cole, Institut Pasteur, Paris, France

FUNDING: The Wellcome Trust

SUMMARY:

Despite the importance of *Mycobacterium tuberculosis* as a major human pathogen, the mechanisms of its pathogenesis are poorly understood. The cell wall of this bacterium is a particularly complex structure, with many genus and species-specific features, some of which have been implicated in virulence. However, little is known about the biosynthesis of cell wall components.

We have been studying this using a genetic approach, initially using the fast growing *Mycobacterium smegmatis*. The core of the cell wall is the same throughout the genus, so this is an appropriate model. *M. smegmatis* strains with defective cell walls were isolated identifying mutants resistant to a range of mycobacteriophages - bacterial viruses which attach to the bacterial wall by binding to specific parts of the cell envelope. The most likely cause of such resistance is the loss of the phages' cell wall receptors. The next stage is to identify the disrupted gene, which will lead on to further genetic and biochemical analysis of the cell wall of the mutant strains.

One mutant was examined, and the defective gene cloned. It was shown to encode an inositol monophosphate phosphatase, which is an enzyme which makes inositol. Inositol is present phosphatidylinositol in mycobacteria, which is required to anchor the major cell wall component, lipoarabinomannan (LAM), in the cell membrane. We showed that a precursor of LAM was present at much lower levels in the mutant. The mutant has altered antibiotic sensitivities, and permeability of the cell wall is altered.

Transferring this strategy to *M. tuberculosis* requires improved mutagenesis procedures, as the current transposon mutagenesis method used for *M. smegmatis* does not work with the pathogen. We have been assessing alternatives, including antisense mutagenesis and modified transposon mutagenesis protocols.

This project ran from February 1993 to May 1997

Publications

1. Parish T. and Stoker NG. (1997) Development and use of a conditional antisense mutagenesis system in mycobacteria. FEMS Microbiol. Lett. 154:151-157.
2. Parish T, Liu J, Nikaido H and Stoker NG (1997) A Mycobacterium smegmatis mutant with a defective inositol monophosphate phosphatase gene homolog has altered cell envelope permeability. J Bacteriol 179:7827-7833

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Functional analysis of the mycobacterial plasmid pAL5000

LSHTM STAFF: Pelle Stolt, Neil Stoker (ITD)

COLLABORATORS: Ken Duncan, Glaxo Research & Development; Valerie Mizrahi, South African Institute for Medical Research, Johannesburg

FUNDING: Glaxo Research & Development

SUMMARY:

Research into the molecular genetics of *Mycobacterium tuberculosis* is difficult because of the slow growth of the bacterium and the need to work in high containment facilities. Added to this is the relatively poor background knowledge in many aspects of their biology. Plasmid vectors are an indispensable tool for this work, yet little is known about the biology of those used. We studied the most widely used plasmid, pAL5000, which was originally isolated from *Mycobacterium fortuitum*, in order to develop more efficient and versatile genetic systems.

We have identified two genes, *repA* and *repB*, in the plasmid which are essential for replication and work in *trans*, in that they can complement another plasmid lacking these genes. *RepB* is a DNA-binding protein, and it binds to two regions on the plasmid - its own promoter, where it presumably represses its own transcription, and another site which is probably the origin of replication. We have carried out site directed mutagenesis of the binding sites to understand the importance of the individual bases, and shown that we can increase the copy number of the plasmid, but only to a limited extent by increasing *repB* transcription.

Using this knowledge we are developing 'delivery systems', which can deliver a transposon (for example) into the cell, remain there as long as we wish in order to allow time for transposition, and then be lost so that the only antibiotic resistant cells are those carrying a transposon in the chromosome. Such a tool would be invaluable in allowing us to isolate mutants of *M. tuberculosis*.

This project started in July 1993 finished in June 1997

Publications

1. Stolt and Stoker (1996) Functional definition of regions necessary for replication and incompatibility in the *Mycobacterium fortuitum* plasmid pAL5000. Microbiology. 142:2795-2802.

2. Stolt, P. and Stoker, N.G. (1996) Protein-DNA interactions in the *ori* region of the *Mycobacterium fortuitum* plasmid pAL5000. J. Bacteriol. 178:6693-6700.

3. Stolt, P. and Stoker, N.G. (1997) Mutational analysis of the regulatory region of the *Mycobacterium* plasmid pAL5000. Nucl. Acids Res. 25: 3840-3846

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Homologous recombination in mycobacteria

LSHTM STAFF: Jason Hinds, Neil Stoker (ITD)

COLLABORATORS: Ken Duncan, Glaxo Research & Development
Richard Stokes, University of British Columbia
Valerie Mizrahi, South African Institute of Medical Research

FUNDING: Glaxo Research & Development, UK

SUMMARY:

A powerful way to understand the function of a gene in *Mycobacterium tuberculosis*, is to inactivate ('knock out') the gene and observe the effect. With the completion of the sequencing of the whole genome, this has become even more important, as we have raw data on thousands of genes, without knowing which are important for pathogenesis. The technology of gene replacement is standard in some other bacterial systems, but has proved to be less easy in *M. tuberculosis*. Gene replacement depends on homologous recombination and one surprising observation in other laboratories has been the high level of non-homologous ('illegitimate') recombination in *M. tuberculosis*.

We used a plasmid construct which allowed us to directly assay the level of homologous recombination. Two parts of an antibiotic resistance gene are cloned, and resistance will only occur if a homologous recombination event occurs to form the whole gene. This was used successfully in *M. smegmatis* to identify conditions under which homologous recombination was enhanced. Using these leads, we have successfully isolated mutants of *M. smegmatis* and *M. tuberculosis*. In addition, we have managed to completely abolish the problem of illegitimate recombination.

This project started in October 1993 and is currently being written up as a PhD thesis

LSHTM TUBERCULOSIS – RELATED RESEARCH

TITLE: The molecular genetics of environmental adaptation in *Mycobacterium tuberculosis*

LSHTM STAFF: Nicola Casali, Neil Stoker (ITD)

COLLABORATORS: Brendan Wren, Dept. of Medical Microbiology, St Bartholomew's Medical School, UK

FUNDING: MRC

SUMMARY:

Bacteria frequently adapt to their environment by means of two-component regulatory systems consisting of a membrane-bound sensor which phosphorylates a cytoplasmic regulator. The regulator then activates and represses sets of genes. Such systems are implicated in the virulence of pathogens, and this project aims to clone and characterize regulator genes from *M. tuberculosis*, and to investigate the roles of these in virulence.

We have been studying genes which we cloned, and which have now been sequenced in the *M. tuberculosis* genome sequencing project. At present, we are concentrating on one gene, *tcrV*, and trying to identify its function.

These genes are of interest not only because they may lead to the identification of new virulence genes, but also because they are good candidate genes for inactivation in developing better vaccines.

This project started in October 1994 and is currently being written up as a PhD thesis.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Inositol metabolism in mycobacteria

LSHTM STAFF: Tanya Parish, Neil Stoker (ITD)

COLLABORATORS: Delphi Chatterjee, Colorado State University, Fort Collins; Hiroshi Nikaido, University of California at Berkeley

FUNDING: The Wellcome Trust

SUMMARY

Tuberculosis remains the infectious disease which causes the greatest mortality world-wide. Mycobacteria are unusual in that unlike most bacteria, inositol is an important molecule, playing a key role in lipoarabinomannan in the cell wall and in the major thiol, mycothiol. We have identified one gene (*impA*) involved in inositol metabolism; the molecular biologist will investigate the function of this and other genes, with the aim of understanding the importance of these inositol-containing molecules in the survival of the fast-growing non-pathogen *Mycobacterium smegmatis*, as well as *Mycobacterium tuberculosis* itself.

This project started in February 1998 and is being funded for three years.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Identification of essential genes of mycobacteria

LSHTM STAFF: Carey Pashley, Neil Stoker (ITD)

COLLABORATORS: Ken Duncan and Ruth McAdam, GlaxoWellcome Research & Development

FUNDING: Glaxo Wellcome Research and Development

SUMMARY:

The identification of new drug targets will allow novel antibiotics to be developed for treatment of tuberculosis. Such targets are likely to be enzymes which are by definition essential for survival of the bacterium. However, gene encoding essential proteins are not identified using standard methods such as transposon mutagenesis, because the mutants of interest die. We are therefore developing techniques for identifying essential mycobacterial genes

This project started in October 1997 and is funded for three years.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Targeted gene inactivation of *M. tuberculosis*

LSHTM STAFF: Tanya Parish, Neil Stoker

COLLABORATORS: Ken Duncan and Ruth McAdam, Glaxo Research & Developmen
Valerie Mizrahi, South African Insitute for Medical Research, Johannesburg

FUNDING: Glaxo Wellcome Research & Development

SUMMARY:

A powerful way to understand the function of a gene in *Mycobacterium tuberculosis*, is to inactivate ('knock out') the gene and observe the effect. Having successfully obtained an kockout mutant of *M. tuberculosis* (see project with Jason Hinds), we are developing the methodology further to see if it works well with other genes. In addition we are streamlining the methodology to reduce the number of colonies that need to be screened (with such a slow-growing organisms, this is a major problem). Initially we are using amino acid biosynthesis mutants, because we know these will not be lethal, and in other organisms can be much less virulent.

This project started in July 1997 and is funded for 18 months.

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LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Dynamics of tuberculous infection and disease.

LSHTM STAFF: Emilia Vynnycky, Paul Fine (ITD)

COLLABORATORS: Martien Borgdorff (KNCV, The Netherlands)
Jan van Embden (RIVM, The Netherlands)

FUNDING: MRC

SUMMARY:

The work has extended the classical modeling work of Sutherland et al to derive age-specific risks of developing primary, endogenous and exogenous disease, the lifetime risks of developing disease, frequency distributions of the incubation period and serial interval and the basic and net reproduction numbers. Analyses to date have been based on data from England and Wales to define the natural history of TB prior to the advent of HIV.

The analyses are being extended to:

1) link with data on clustering of DNA fingerprints from tuberculosis patients observed in the Netherlands during the period 1993-6, in collaboration with Martien Borgdorff (KNCV) and Dr van Embden (RIVM).

2) contrast the different patterns of tuberculous disease observed in developed and developing countries, using data from Malawi (and possibly South India).

3) explore the effect of the HIV epidemic on tuberculosis trends.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A novel microbiological approach to detection and drug susceptibility-testing of *Mycobacterium tuberculosis*

LSHTM STAFF: Zubaida Al-Suwaidi (ITD)

COLLABORATORS: Stuart Wilson, Francis Drobniewski, Mycobacterium Reference Unit, PHLS Dulwich Hospital

FUNDING: Government of Qatar, Public Health Laboratory Service.

SUMMARY:

Conventional culture-based drug susceptibility tests for *Mycobacterium tuberculosis* are slow, taking 10-21 days from culture isolates or 6-12 weeks from patient samples. Alternative genotypic tests have an advantage in that they are rapid and sensitive and are useful in the detection of simple drug resistance genotypes such as resistance to rifampicin. They have not, however, been successfully applied to more complicated mechanisms of resistance such as to that of isoniazid. In addition genotypic tests are often complicated and expensive which limits their use in low-income countries which have a high burden of tuberculosis.

To address this problem we have been developing an idea, the PhaB (phage amplified biologically) assay which was conceived by Stuart Wilson in 1992 whilst he was working at the School. The PhaB assay is a biological assay for viable *Mycobacterium tuberculosis*. It is simple, inexpensive and extremely sensitive. The principle is straightforward. The assay depends on the ability of viable Mycobacterium tuberculosis to protect infecting mycobacteriophage (viruses which are specific for mycobacteria) from chemical inactivation and to support the replication of the infecting phage. Drug susceptibility of an isolate is tested by incubating the isolate in the presence of drug. If the isolate is susceptible to the drug it becomes non-viable and cannot protect or host replication of the mycobacteriophage. If, on the other hand, the bacilli are resistant to the drug the mycobacteriophage are protected and replicate. The replicated progeny are subsequently detected by growth on a rapidly-growing mycobacterial host. The assay is sensitive, able to detect as few as 100 bacilli and is rapid, taking as few as 2-3 days to perform. Ultimately, the assay is applicable to direct drug susceptibility-testing of patient samples and direct detection of *Mycobacterium tuberculosis* in patient samples.

We have performed a small-scale evaluation of the PhaB assay for the drug susceptibility-testing of cultured isolates using isolates from the MRU. The results correlated closely with those of conventional methodologies and correctly identified multi-drug resistant strains of Mycobacterium tuberculosis. A larger evaluation is underway on isolates and primary patient samples.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Models of cell mediated immunity to *Mycobacterium tuberculosis*

LSHTM STAFF: Debbie Smith, Corine Bronke, Greg Bancroft (ITD)

COLLABORATORS:

FUNDING: GLAXO ActionTB Initiative

SUMMARY:

As part of the GLAXO Action Tuberculosis Programme, we are investigating the mechanisms of cell mediated immunity involved in protection of the host against *Mycobacterium tuberculosis*. Our approach is to study infection with *M. bovis* BCG, *M. avium* and *M. tuberculosis* in experimental models to identify how immunodeficiency predisposes the host to lethal injection.

This work aims to understand the mechanisms of phagocyte recruitment and activation at sites of tissue granulomatous response against Mycobacteria. This process is crucial in determining the control of bacterial replication by the host and in turn affects the pathology of the disease, leading either to caseating necrosis, erosion of adjacent airways and transmission to other individuals or in contrast, containment of the initial bacterial focus and localised fibrosis. In particular, the relative contribution of NK cell versus T cell derived cytokines and the importance of chemotactic factors (such as MIP-1α etc.) in recruitment of phagocytes to foci of bacterial replication have not been adequately defined.

In an experimental model of infection with *M.avium* we have now observed that even in the absence of T cells, granulomatous responses can still be generated during mycobacterial infection. Recruitment of cells to bacterial foci can be blocked by prior neutralisation of the pro-inflammatory cytokines TNFα or IFNγ. Additionally, by using RT-PCR for cytokine production *in situ* we have shown that infection with M avium causes induction of pro inflammatory cytokines including TNF, IFNg, IL-1, IL-12, MIP-1α and IL-6 in infected tissues. Treatment with neutralising antibodies to either TNFα or IFNγ reduced *in situ* mRNA levels for these cytokines. This early IFNg secretion of NK cells may be essential in the priming of macrophages for enhanced recruitment and activation at sites of mycobacterial growth.

These studies have been extended to investigate the spectrum of chemokines, small inducible secreted proinflammatory cytokines responsible for cell specific migration and their receptors. Thus we have shown that despite the absence of T cells SCID models generate potent and complex chemokine and chemokine receptor mRNA responses to mycobacteria which are qualitatively similar to those seen in immunocompetent mice.

In conclusion, these results using *M.avium,* BCG and *M.tuberculosis* have identified a novel role for NK cell derived cytokines in controlling phagocyte recruitment in infected tissues and demonstrated that glucocorticoids have powerful regulatory effects on the balance of cytokines secreted by host phagocytes after ingestion of Mycobacteria.

In addition, Mycobacteria are potent activators of chemokine and chemokine receptors in infected tissues. Our goals are to now identify the mechanisms by which this upregulation takes place and the effect of these responses on chemokine biology in vivo.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Cell envelope biosynthesis in mycobacteria

LSHTM STAFF: Paul Wheeler, Paul Anderson (ITD)

COLLABORATORS: Neil Freeman, Keith Brinded, Paul Hissey (Glaxo Wellcome)
Ralph Schwarz (University of Marburg)
Delphi Chatterjee, G.S. Besra (Colorado State University)

FUNDING: GlaxoWellcome Action-TB

SUMMARY:

The distinct lipid-rich cell envelope of *Mycobacterium tuberculosis* is an important defence for the bacterium against a hostile environment, and it is also important in stinulating a self-damaging reponse from the human immunew system. This project sets out to study the action of isoniazid (a first-line anti-TB drug) on lipoarabinomannan (LAM; a major component of the mycobacterial cell wall). The work focuses on the enzymes which build up the LAM, in particular the mannosyltransferases.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: Studies on BCG vaccination

LSHTM STAFF: Steve Bennet (ITD)

COLLABORATORS: Arnaud Marchant, Melanie Newport

FUNDING: MRC

SUMMARY

1. A controlled trial of BCG vaccination at birth, 2 months and 4 months to determine the effect of timing of vaccination on immune responses.

2. A study of immune responses to BCG vaccination in newborn twins, in order to determine the genetic contribution to these responses.

LSHTM TUBERCULOSIS - RELATED RESEARCH

TITLE: A randomised double blind controlled safety and immunogenicity study of subcutaneous recombinant human interleukin-12 (RHIL-12) in patients with pulmonary tuberculosis in The Gambia

LSHTM STAFF: Shabbar Jaffar (ITD)

COLLABORATORS: Dr T Corrah *et al* MRC labs

FUNDING: MRC / Genetics Institute USA.

SUMMARY:

Reducing the length of time required for treatment of tuberculosis is a major goal that might be achieved by adding immunotherapy to antibacterial therapy. This study will investigate whether rhIL-12 is an appropriate immuno-stimulant in patients with tuberculosis. It is proposed to proceed to a randomised efficacy trial in patients with pulmonary TB.